510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY TEMPLATE

A. 510(k) Number:

k123880

B. Purpose for Submission:

New Device

C. Measurand:

Anti-centromere protein B anti-nuclear antibodies

D. Type of Test:

Semi-Quantitative, Chemiluminescent Immunoassay (CIA)

E. Applicant:

INOVA Diagnostics, Inc.

F. Proprietary and Established Names:

QUANTA Flash Centromere Reagents

QUANTA Flash Centromere Calibrators

QUANTA Flash Centromere Controls

G. Regulatory Information:

1. Regulation section:

   21 CFR §866.5100, Antinuclear Antibodies Immunological Test System

   21 CFR §862.1150, Calibrator

   21 CFR §862.1660, Quality Control Material (Assayed and Unassayed)

2. Classification:

   Class II – Assay and Calibrator

Class I – Control

3. Product code:

   LJM –Antinuclear antibody (enzyme-labeled), antigen, controls

   JIX – Calibrator, Multi-Analyte Mixture

   JJX – Single (Specified) Analyte Controls (Assayed and Unassayed)

4. Panel:

   Immunology (82) (Assay)

   Chemistry (75) (Calibrator and Controls)

H. Intended Use:

1. Intended use(s):

   QUANTA Flash Centromere is a chemiluminescent immunoassay for the semi-quantitative determination of IgG anti-centromere protein B autoantibodies in human serum. The presence of anti-centromere protein B autoantibodies is used as an aid in the diagnosis of Systemic Sclerosis, in conjunction with clinical finding and other laboratory tests.

   QUANTA Flash Centromere Calibrators are intended for use with the QUANTA Flash Centromere Reagents for the determination of IgG anti-centromere protein B autoantibodies in human serum. Each calibrator establishes a point of reference for the working curve that is used to calculate the unit values.

   QUANTA Flash Centromere Controls are intended for use with the QUANTA Flash Centromere Reagents for quality control in the determination of IgG anti-centromere protein B autoantibodies in human serum.

2. Indication(s) for use:

   Same as Intended Use

3. Special conditions for use statement(s):

   For prescription use only

4. Special instrument requirements:

   BIO-FLASH® Instrument System (k083518)

I. Device Description:

The QUANTA Flash Centromere Reagent cartridge contains one reagent cartridge with sufficient material for 50 tests. The QUANTA Flash Centromere reagent cartridge contains 4 reagent tubes:

- Centromere coated to paramagnetic beads in a buffered solution containing protein stabilizers and preservative;
- Assay buffer – phosphate-buffered saline solution containing Tween 20, protein stabilizers and preservatives;
- Tracer IgG – Isoluminol labeled anti-human IgG antibodies in a buffered solution containing protein stabilizers and preservative;
- Centromere Reagents include Resuspension Buffer and a transfer pipette for re-suspending the beads.

The QUANTA Flash Centromere Calibrator set includes two calibrators (Calibrator 1 and Calibrator 2). These are barcoded tubes containing 0.3 mL pre-diluted, ready-to-use reagent. Calibrators contain human antibodies to centromere in buffer. They are sold separately.

The QUANTA Flash Centromere Controls contain two vials (a Negative and a Positive) containing human antibodies to centromere in buffer, protein stabilizers and preservatives. They are sold separately.

The following additional reagents are required for the test and supplied separately; BIO-FLASH System Rinse, BIO-FLASH Triggers, and BIO-FLASH Cuvettes.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   QUANTA Lite Centromere ELISA

2. Predicate 510(k) number(s):

   k003959

3. Comparison with predicate:

Similarities		
Item	Device	Predicate
Intended Use	QUANTA Flash Centromere is a chemiluminescent immunoassay for the semi-quantitative determination of IgG anti-centromere protein B autoantibodies in human serum. The presence of anti-centromere protein B autoantibodies is used as an aid in the diagnosis of connective tissue diseases including systemic sclerosis in conjunction with clinical finding and other laboratory tests.	Same
Assay Type	Semi-quantitative immunoassay	Same
Analyte Detected	Human anti-Centromere autoantibodies (IgG)	Same
Sample Matrix	Serum	Same
Cut-off between positive and negative	20 units	Same

Differences		
Item	Device	Predicate
Assay Methodology	Chemiluminescent Immunoassay utilizing magnetic particles	Enzyme-linked Immunosorbent Assay
Conjugate	Isoluminol conjugated monoclonal anti-human IgG antibodies	Horse radish peroxidase conjugated goat anti-human IgG antibodies
Signal Detected	Luminescence (visible light)	Absorbance at 450nm
Calibration and unit calculation	Instrument specific working curve based off a 6 point lot specific master curve used for unit calculations; stored on the instrument for 73 days for Centromere assay.	Single point calibrator run each time the assay is run
Centromere antigen	Recombinant CENP-B antigen	Recombinant CENP-A and CENP-B antigen

K. Standard/Guidance Document Referenced (if applicable):

- CLSI EP5-A2, Evaluation of Precision Performance of Quantitative Measurement Methods.
- CLSI EP17-A, Protocols for Determination of Limits of Detection and Limits of Quantitation.
- CLSI EP6-A, Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach.
- CLSI H18-A4, Procedures for Handling and Processing Blood Specimens.

L. Test Principle:

The assay technology is similar to other solid phase indirect immunosorbent assays. The assay solid phase is paramagnetic beads centromere antigens coated onto paramagnetic beads. Patient samples are loaded into sample racks which are then placed into the sample carousel of the instrument. A patient's serum is diluted by the instrument with sample dilution buffer in a disposable cuvette. A small amount of the diluted sample is combined with assay buffer and antigen coated beads in a second cuvette, and mixed. This reaction cuvette is incubated at 37°C. The cuvette is then exposed to a small magnet that holds the beads in place, the liquid is aspirated, and the beads are re-suspended as system rinse is added to the cuvette and the magnet is removed. This wash cycle is repeated one more time. During the third wash, no system rinse is added after the aspiration step.

The detecting reagent is an isoluminol-conjugated anti-human IgG monoclonal antibody. After the third wash, isoluminol conjugated monoclonal anti-human IgG is added to the beads in the cuvette, and mixed. Again, the cuvette is incubated at 37°C. Then, three wash steps, as described above, are performed on the beads. In the fourth wash step, no liquid is added to the beads after the aspiration. The cuvette is then placed in a light-tight luminometer and the beads are exposed to a base and an oxidizing agent. These two reagents, or "Triggers", cause the isoluminol to produce a flash of visible light. The light produced from this reaction is measured as Relative Light Units (RLU) by the BIO-FLASH optical system. The RLU are proportional to the amount of autoantibodies bound to the antigen on the beads.

The QUANTA Flash Centromere assay utilizes a predefined lot specific Master Curve that is loaded into the instrument through the reagent cartridge barcode. Based on the results of two calibrators, a working curve is created, which is used to calculate chemiluminescent units (CU) from RLU. The calibrators are not included as part of the QUANTA Flash Centromere Reagent Kit.

M. Performance Characteristics (if/when applicable):

1. <u>Analytical performance:</u>

   a. Precision/Reproducibility:

Precision of the QUANTA Flash Centromere assay was evaluated by running eight patient samples in duplicates, twice a day, for 20 days on one reagent lot (total of 80 replicates per sample). Controls were run as quality control during each run. The study results are summarized in the table below:

		Within-Run		Between-Run		Between-Day		Total	
Sample	Mean (CU)	SD	% CV	SD	% CV	SD	% CV	SD	% CV
A	6.2	0.2	2.6%	0.2	3.0%	0.3	5.4%	0.4	6.7%
B	13.5	0.5	3.7%	0.3	2.1%	0.7	4.9%	0.9	6.5%
C	16.6	0.9	5.2%	0.9	5.6%	0.9	5.5%	1.6	9.4%
D	16.8	0.8	4.9%	0.9	5.3%	1.0	5.9%	1.6	9.3%
E	22.9	0.9	3.7%	0.5	2.2%	0.9	4.0%	1.4	5.9%
F	60.9	2.0	3.2%	1.3	2.1%	3.4	5.5%	4.1	6.7%
G	103.3	5.7	5.6%	0.4	0.4%	4.2	4.1%	7.1	6.9%
H	197.9	13.2	6.7%	0.0	0.0%	11.9	6.0%	17.7	9.0%
I	321.4	24.5	7.6%	0.0	0.0%	27.8	8.7%	37.1	11.5%
J	463.2	33.6	7.3%	0.0	0.0%	22.9	4.9%	40.7	8.8%

The lot-to-lot reproducibility of the assay was evaluated by testing samples with concentrations across the assay range. Five samples each in low, moderate, and high concentrations were tested in duplicate in three separate assay lots:

Group	Sample Range (CU)	Average %CV of Group	Range of % CV between Lots
Low	16.8 – 35.2	5.8%	1.0 – 7.3%
Moderate	60.2 – 121.3	5.0%	2.3 – 11.5%
High	214.9 – 568.9	7.1%	1.2 – 11.6%

b. Linearity/assay reportable range:

The analytical measuring range (AMR) of the assays is defined by the lowest and highest points on the Master Curve (3.4 – 708.9 CU). The linearity across this range was determined by diluting a high positive samples with a negative samples by the dilution scheme recommended in CLSI EP6-A. To cover the entire range several dilution series (starting at different points on the curve) were generated and tested. The expected value was plotted against the observed value, and linear regression analysis was performed to get slope, intercept and R^2 values. The results are summarized in the table below.

Hook effect:

There is no hook effect observed for the Centromere assay.

Sample	Test Range (CU)	Slope (95% CI)	Y-intercept (95% CI)	R^2	Percent Recovery
8	4.1 – 20.3	1.01 (0.98 –1.03)	0.25 (-0.04 –0.56)	1.00	99.8 –105.9%
4	6.4 –63.7	0.98 (0.96 –1.00)	1.89 (1.15 –2.64)	1.00	80.3 –100.5%
9	10.6 –106.1	1.01 (0.99 – 1.02)	-1.24 (-2.41 –-0.07)	1.00	99.1 –109.5%
2	12.7 –127.4	0.99 (0.97 –1.01)	2.27 (0.89 –3.64)	1.00	80.8 –101.0%
5	24.4 –244.4	0.99 (0.97 –1.01)	-0.20 (-3.18 –2.78)	1.00	89.7 –106.6%
7	90.4 –632.7	1.04 (1.01 –1.08)	-25.39 (-38.69 –-12.09)	1.00	86.1 –100.4%
All	4.1 – 632.7	1.01 (1.00 –1.01)	-1.10 (-2.61 –0.41)	1.00	80.3 –109.5%

To assess the auto-rerun capability of the instrument, two samples greater than the highest point of the reportable range were run with the auto-rerun feature enabled. The manual dilution and the instrument re-run result variations were between -6% and 9% for the samples tested.

c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*

Traceability and Value Assignment: There is no recognized reference standard for the measurement of anti-Centromere antibodies. Assay calibrators and controls are traceable to in-house standards that are used to create the lot-specific master curve for the QUANTA Flash Centromere assay. Calibrators and controls are manufactured by diluting human serum that contains high titer of anti-centromere antibodies with a buffer stabilizers and preservative. The human serum is obtained from commercial sources and it is tested for markers of infectious substances. The target CU value is achieved through trial dilutions on a small scale. Once a dilution is selected, the calibrators and controls are bulked, tested, and adjusted. Upon completion of the manufacturing process, the calibrators and controls are tested on at least two instruments, on at least two lots, of reagent cartridge in replicates of 10 to determine final value assignment. Calibrators and control materials are specified in the labeling but are not supplied with the assay.

Kit Stability: The claimed stability of one year at 2 – 8°C for unopened kits is based on accelerated studies. Real-time stability is ongoing and the results are shown below. The BIO-FLASH software monitors the expiration dates of the onboard cartridges, as well as the reagent cartridge lots.

Product	Real time testing	Specifications met
QUANTA Flash Centromere Reagents	10 months	yes
QUANTA Flash Centromere Calibrators	12 months	yes
QUANTA Flash Centromere Controls	12months	yes

Opened kit components are stable as follows:

Reagent Cartridge and beads	73 days
Calibrators	8 hours total use time onboard; up to 4 uses
Controls	10 min per use, up to 2.5 hours total onboard or up to 15 uses (whichever is fulfilled first)

The sponsor recommends that serum samples be stored according to the guidelines in CLSI H18-A4 and cautions against using samples that are microbially contaminated, heat-treated, or contain visible particulates.

d. Detection limit:

Limit of Blank (LoB): 30 samples were run at least twice, in replicates to obtain at least 60 measurements using Immunoglobulin-stripped serum as the analyte-free material. The RLU value of these samples was lower than the bottom limit of the four parameter logistic curve that the instrument used to calculate CU, and therefore cannot be converted into CU. The Limit of Blank is claimed to be < 3.4 CU for Centromere antigen.

Limit of Detection (LoD): Five sera for the Centromere CIAs were serially diluted until the RLU values no longer showed a decreasing trend. Serum samples were diluted to a concentration that was between the LoB and approximately 4 x LoB. These five samples were run in 4 replicates for 4 days; 2 days on 1 instrument and 2 more days on another instrument to obtain 16 measurements per sample for at least 80 measurements. The Limit of Detection for the assay is below the lower limit of the analytical measuring range (3.4 CU).

e. Analytical specificity:

Endogenous Interferents:
Sera with Centromere antibodies of different concentrations (negative, positive and around the cut-off) were spiked mixed with known quantities of bilirubin (10, 5 or 2.5 mg/dL), hemoglobin (200, 100 or 50 mg/dL), cholesterol (224.3, 112.2 or 56.0 mg/dL), triglycerides (1000, 500 or 250 mg/dL) or rheumatoid factor (RF) (about 500, 300 or 100 IU/mL). Each

sample was tested in triplicate and the recovery was calculated by comparing to control samples spiked with the same volume of diluents. No interference (≤ 15%) was detected in the samples up to the concentrations listed in the table below:

Potential Interfering Compound	Maximum Concentration	Range of % Recovery
Bilirubin	10 mg/dL	91 – 100%
Hemoglobin	200 mg/dL	89 – 100%
Triglycerides	1000 mg/dL	87 – 100%
Cholesterol	224.3 mg/dL	87 – 100%
Rheumatoid factor	500 IU/mL	100 – 115%

Analytical Cross-reactivity:
A potential cross-reactivity of the QUANTA Flash Centromere CIA with other autoantibodies was evaluated by testing 51 SLE samples known to have high levels of various other antibodies (i.e., anti-dsDNA, anti-CCP, anti-chromatin, anti-DGP, etc.). All samples were negative by the QUANTA Flash Centromere CIA.

f. Assay cut-off:

The assay cut-off is 20 CU. Results <20 CU should be reported as negative and results ≥20 CU should be reported as positive.

2. Comparison studies:

a. Method comparison with predicate device:

Three hundred and fifty eight (358) samples were tested by QUANTA Lite Centromere ELISA and QUANTA Flash Centromere CIA. Summarized in the table below, the positive and negative agreements are 84.5% and 99.3%, respectively and the overall agreement is 95.4%. Of the 358 samples, only 107 were within the analytical measuring range:

		Centromere ELISA			Percent Agreement (95% CI)
		Positive	Negative	Total	
QUANTA Flash Centromere	Positive	41	1	42	Pos. Agree = 85.4% (72.2-93.9%)
	Negative	7	58	65	Neg. Agree = 98.3% (90.9-100.0%)
	Total	48	59	107	Total Agree = 92.5% (85.8-96.7%)

To increase the number of samples in the analytical measuring range of both

assays, an additional 27 contrived samples were tested. Contrived samples were made by diluting clinically diagnosed systemic sclerosis samples that are positive on the QUANTA Flash Centromere assay with normal serum to desired reactivity. Total number of samples for this second method comparison was 134 and results are summarized below:

		Centromere ELISA			Percent Agreement (95% CI)
		Positive	Negative	Total	
QUANTA Flash Centromere	Positive	53	7	60	Pos. Agree = 84.1% (72.7-92.1%)
	Negative	10	64	74	Neg. Agree = 90.1% (80.7-95.9%)
	Total	63	71	134	Total Agree = 87.3% (80.5-92.4%)

b. Matrix comparison:

Not applicable since human serum is the only claimed specimen matrix.

3. Clinical studies:

a. Clinical sensitivity and specificity:

To determine the sensitivity and specificity of the QUANTA Flash Centromere assay, a total of 831 samples from patients diagnosed with systemic sclerosis and various disease controls were tested. No demographic information was available for the samples. See below for the number of subjects for each disease tested in this analysis. Samples from normal blood donors were excluded from the calculations. The sensitivity and specificity results are summarized below:

All Patients (n=831)		**Diagnosis – Systemic Sclerosis**			**Percent Agreement (95% confidence interval)**
		Positive	Negative	Total	
QUANTA Flash® Centromere CIA	Positive	32	6	38	Sensitivity = 23.4% (16.6-31.3%)
	Negative	105	688	793	Specificity = 99.1% (98.1-99.7%)
	Total	137	694	831	

The table below shows the results for each clinical subgroup and each analyte.

Cohort	n=	No (%) pos
Systemic sclerosis (SSc)	137	32 (23.4 %)
Other Inflammatory Diseases	622	6 (1.0%)
Systemic lupus erythematosus (SLE)	170	1 (0.6%)
Rheumatoid arthritis	92	1 (1.1%)
Inflammatory bowel disease	49	0 (0.0%)
Crohn`s disease	27	0 (0.0%)
Ulcerative colitis	30	0 (0.0%)
Multiple sclerosis	19	0 (0.0%)
Hashimoto`s thyroiditis	33	0 (0.0%)
Grave`s disease	58	0 (0.0%)
Interstitial cystitis	40	0 (0.0%)
Asthma	27	1 (3.7%)
Other Disease Controls*	77	3 (4.6%)
Infectious Diseases:	72	0 (0.0%)
Hepatitis C virus infection	24	0 (0.0%)
Hepatitis B virus infection	31	0 (0.0%)
Human immunodeficiency virus infection	8	0 (0.0%)
Syphilis	9	0 (0.0%)

*non-systemic autoimmune disease, diagnosis pending (30), Osteoarthritis (14), Late Onset SLE (6), Polymyalgia Rheumatica (6), Connective Tissue Disease (5), Raynaud's Disease (3), SLE/Raynaud's Overlap (2), Gout (2), Psoriasis Arthritis (2), Primary Biliary Cirrhosis (1), Rosacea (1), Sakroiliitis (1), Fibromyalgia (1), Carcinoma of the Colon (1), QT syndrome (1), Incomplete CREST (1)

b. Other clinical supportive data (when a. is not applicable):

Not applicable.

4. Clinical cut-off**:**

There is no clinically accepted cutoff defined for this analyte.

5. Expected values/Reference range:

400 samples from random blood donors were tested of which four samples (1.0%) were positive. The sample average value was 4.0 CU; 95% of the samples had results between 3.5 – 4.5 CU.

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

O. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.